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Effects of elevated atmospheric CO_2 on two southern forest diseases

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Abstract Research into the effects of rising atmospheric carbon dioxide (CO_2) on plant diseases remains limited despite the economic importance of this subject. Loblolly pine (*Pinus taeda*) seedlings were exposed to ambient and twice ambient levels of atmospheric CO_2 prior to inoculation with the fusiform rust fungus (the obligate pathogen *Cronartium quercuum* f.sp. *fusiforme*, CQF) or the pitch canker fungus (the facultative pathogen *Fusarium circinatum*, FC). Additionally, northern red oak seedlings (*Quercus rubra*; an alternate host of CQF) were exposed to ambient or elevated levels of atmospheric CO_2 prior to inoculation with CQF. In all cases, disease incidence (percent of plants infected) and disease severity (proportion of each plant affected) were determined; with the oak seedlings, the latent period (time to sporulation) was also monitored. In general, disease incidence was decreased by exposure to elevated CO_2. This exposure also increased the latent period for CQF on oak seedlings. In no instance did exposure to elevated CO_2 affect disease severity. This research demonstrated that plants may benefit from exposure to the increasing concentration of CO_2 in the atmosphere through decreases in fungal disease incidence.

Keywords Atmospheric carbon dioxide · Fusiform rust · Pitch canker · *Cronartium quercuum* f.sp. *fusiforme* · *Fusarium circinatum* · Loblolly pine · *Pinus taeda* · Northern red oak · *Quercus rubra*

Introduction

The concentration of carbon dioxide (CO_2) in the atmosphere is rising, due primarily to fossil fuel combustion and deforestation, and is projected to double pre-industrial levels within this century (Keeling and Whorf 1994). Plant responses to elevated CO_2 are well

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documented (Kimball 1983) and may profoundly influence plant–microbe interactions; however, limited experimentation has occurred to date. Thus, the manner in which increases in atmospheric CO_2 will affect the major diseases of the world's plants are largely unknown. This is of vital importance in that each year billions of dollars in plant yield are lost to diseases and millions more are spent managing these pests (Agrios 1988).

For most plant species, experimentally doubling atmospheric CO_2 has been shown to increase photosynthesis (Amthor 1995) and water use efficiency (Rogers and Dahlman 1993) which leads to increased plant growth. On average, biomass of plants with a C_3 photosynthetic pathway is increased by almost 40% under elevated CO_2 (Poorter 1993); this is also true for most woody species (Ceulemans and Mousseau 1994). Increasing plant size would provide more surface area for infection and the incidence of disease may increase. However, the larger plants may tolerate higher levels of infection without subsequent reductions in yield.

In addition to increases in plant biomass, growth in elevated CO_2 often results in altered tissue chemistry. Increased carbohydrate content (Yelle et al. 1989; Runion et al. 1999) and reductions in tissue nutrient concentration (especially nitrogen; Norby et al. 2001), leading to increases in tissue C:N ratios (Mellilo 1983; Torbert et al. 2000), are commonly observed in plants grown under elevated CO_2. Other alterations in plant tissue chemistry that have been observed under elevated CO_2 include changes in lignin quantity and higher lignin:N ratios (Runion et al. 1999; Norby et al. 2001), and increased levels of defense compounds such as tannins and phenolics (Pritchard et al. 1997; Entry et al. 1998; Runion et al. 1999).

Pathogens—like plants—require water, nutrients, and a source of energy for growth, reproduction, and to carry out the processes involved in infection. Increased plant water use efficiency under elevated CO_2 could result in increased disease incidence and/or severity. Similarly, increased carbohydrate content of plant tissues under elevated CO_2 could provide additional substrate for increased growth and reproduction of pathogens and result in increased disease incidence and/or severity. Conversely, lower tissue N concentrations or increases in plant defense compounds might have the opposite effect.

In addition to alterations in plant tissue chemistry, changes in morphology have been noted to occur in plants grown under elevated CO_2. Extra layers of leaf epidermal cells (Thomas and Harvey 1983) could make it more difficult for pathogens to penetrate their hosts. Changes in the quantity and quality of foliar epicuticular waxes (Graham and Nobel 1996; Prior et al. 1997) may make adhesion and penetration more or less difficult, depending on the specific nature of these alterations.

The impacts of these myriad CO_2-induced changes on plant diseases will undoubtedly vary depending upon the host, the pathosystem of interest, and the specific environmental conditions in which they are grown. Thompson et al. (1993) found that, when wheat (*Triticum aestivum* L. cv. Cerco) seedlings received adequate water, leaf N content declined, leaf water content was unchanged, and infection by the powdery mildew fungus (*Erysiphe graminis* DC.) was reduced under high CO_2; however, under water limiting conditions leaf N was unchanged, leaf water content increased, and mildew infection increased under high CO_2. In further work, Thompson and Drake (1994) related lower severity of a foliar rust disease (caused by *Puccinea sparganioides* Ellis & Barth.) of a C_3 sedge (*Scirpus olneyi* Grey.) under elevated CO_2 to reductions in leaf N, but attributed an increase in disease severity (fungus undetermined) for a C_4 grass [*Spatina patens* (Aiton) Muhl.] under elevated CO_2 to increased leaf water content. Hibberd et al. (1996) reported that penetration (a measure of incidence) of barley (*Hordeum vulgare* L. cv. Blenheim) by the powdery mildew fungus, *Erysiphe graminis* (DC.), was reduced under elevated CO_2 due to changes in host leaves (i.e., increased epicuticular waxes, papillae, and silicon

accumulation at the sites of penetration); however, growth of established fungal colonies (a measure of severity) was increased under elevated CO_2, which they related to increased leaf carbohydrate status under elevated CO_2.

Given the paucity of data, it is difficult to draw conclusions regarding the potential of elevated CO_2 to affect plant–microbe interactions. Further, increasing concentrations of atmospheric CO_2 will likely have vastly different effects on different host-pathogen systems. However, generalities regarding the effects of CO_2 on host–pathogen interactions can be theorized using knowledge of ecophysiological differences among pathosystems (Runion et al. 1994) and it was hypothesized that elevated atmospheric CO_2 will have differing effects on diseases caused by obligate versus facultative pathogenic fungi. Obligate pathogens have a more intimate relationship with their host and must have the host to survive. Facultative pathogens, on the other hand, live saprophytically and generally result in disease (or tend to be more severe) under conditions of plant stress such as low nutrition or water (Marschner 1986).

Incidence of disease is controlled, in large part, by growth of the host. Increases in plant size under elevated CO_2 should provide more surface area for infection by both obligate and facultative pathogens. However, if supplied with adequate nutrition and water, these larger plants should be better able to resist infection by facultative pathogens. Therefore, high CO_2-induced increases in plant size will increase incidence of diseases caused by obligate pathogens while incidence due to facultative pathogens will decrease or remain unaffected.

Disease severity is primarily affected by host phytochemistry (carbohydrates and N). Facultative pathogens possess their own system for generating energy using components from plant cells they have killed and are less dependent on the host's photosynthetic system. Obligate pathogens, at least in the early stages of infection, rely on assimilates supplied by living cells and result in limited alteration of the host's photosynthetic system (Kosuge 1978). Additionally, even though plant tissue N concentration is often lower under elevated CO_2, this may be due to a "dilution effect" resulting from the plants being larger and total plant N content can be unaffected or even increased. Therefore, it was hypothesized that—as with disease incidence—severity of disease from obligate pathogens will increase under elevated CO_2 (given adequate water and nutrition), while that of facultative pathogens will decrease or remain unaffected.

To test these hypotheses, one species of southern pine and one species of oak were grown under ambient or twice ambient levels of atmospheric CO_2. These plants were then inoculated with either an obligate or a facultative fungal pathogen which are known to cause important diseases in the Southeastern US.

Materials and methods

Fusiform rust study: pine

Seeds of loblolly pine (*Pinus taeda* L.) from a mixed lot of half-sib families were obtained from an Alabama forest seed orchard, scarified by soaking in 3% hydrogen peroxide for 12 h, washed under running tap water for 10 min, and air dried for 30 min on sterile paper towels. Seeds were then placed into germination flats (50 cm × 25 cm) containing a peat based general purpose growing medium (PRO-MIX Bx, Premier Horticulture Inc., Quakertown, PA 18951) in a temperature controlled glasshouse. After dropping their seed coats, seeds were transplanted into plastic containers (10 × 10 × 36 cm) containing a

3:1:1 (v/v/v) mixture of soil, sand, and peat moss at one seedling per container. Nutrients were added at the time the growth medium was prepared. Nitrogen was added as sulphur-coated urea at 0.2 mg N g^{-1} soil mixture. Other nutrients were supplied in the form of sulphur-coated potassium at 0.04 mg K g^{-1} soil mixture and Micromax Plus which contains P at 0.14 mg g^{-1}, Ca at 0.57 mg g^{-1}, Mg at 0.28 mg g^{-1}, and S at 0.05 mg g^{-1} soil mixture, plus a complete complement of micronutrients (Scotts-Sierra Horticultural Products Company, Marysville, OH). Throughout the study seedlings received deionized water as needed.

Seedlings were held in the glasshouse over the winter. Uniform sized seedlings were selected for placement into open top field chambers for carbon dioxide (CO_2) exposure in early spring. The open top field chamber system is located at the soil bin facilities at the USDA-ARS National Soil Dynamics Laboratory, Auburn, Alabama. The bin used for this experiment is 6 m wide and 76 m long and was modified for container studies. Modifications consisted of installing a geomembrane liner (20 mil) and gravel drain system to ensure a good working surface and drainage system for container studies. Open top chambers (Rogers et al. 1983), encompassing 7.3 m^2 of ground surface area, were used to maintain (24 h per day) target CO_2 concentrations of $\sim$360 µmol mol^{-1} (ambient) or $\sim$720 µmol mol^{-1} (elevated) using a delivery and monitoring system described by Mitchell et al. (1995).

The bin was divided into five blocks and each CO_2 treatment was randomly assigned to one open top chamber within each block (total chambers = 10). The experimental design was a randomized complete block design, with blocks occurring along the length of the soil bin.

Seedlings (13 per chamber) were exposed to the two concentrations of atmospheric CO_2 for 6 weeks, at which time they had developed new succulent apical growth (candles). Seedlings were removed from open top chambers, measured (height and groundline diameter), and ten per chamber were inoculated with basidiospores of the fusiform rust fungus [*Cronartium quercuum* (Berk.) Miyabe ex Shirai f.sp. *fusiforme* (Hedgc. & Hunt) Burdsall & G. Snow (CQF)]. The inoculum was prepared by suspending naturally infected northern red oak (*Quercus rubra* L.) leaves over sterile water in enclosed plastic boxes (15 × 10 × 10 cm). The water was decanted until a spore suspension of 5×10^3 spores ml^{-1} was achieved. This spore suspension was placed into a sterile glass mist atomizer bottle and 100 seedlings were sprayed to runoff. Seedlings were then held in a growth chamber at 25°C and 100% relative humidity for 24 h at which time they were returned to the open top chambers. An additional three seedlings per chamber were sprayed to runoff with sterile distilled water, to serve as controls, held as with the inoculated seedlings and returned to the open top chambers. Seedlings were held within the two CO_2 treatments for 7 months at which time they were assessed for symptoms of disease. The percentage of seedlings infected was recorded as were the number and size (length and diameter) of each fusiform rust gall. This study was repeated the following year.

Fusiform rust study: oak

Northern red oak acorns, collected from a local source, were soaked in sterile water for 24 h, placed onto sterile moist filter paper in germination trays (75 × 50 cm), and held in a growth chamber (25°C) until germination (5–10 days). Germinated seeds were transplanted into plastic containers (10 × 10 × 36 cm) containing a 3:1:1 (v/v/v) mixture of soil, sand, and peat moss at one seed per container. Uniform size seedlings were selected from among the total for exposure to CO_2 treatments. Nutrients and water were applied as with the loblolly pine study detailed above.

Seedlings were immediately placed into the same open top CO_2 exposure field chambers described above, utilizing the same experimental design. Seedlings were exposed to the two concentrations of atmospheric CO_2 for 4 weeks, at which time they had 3–5 fully developed new succulent leaves. Seedlings were removed and inoculated with aeciospores of the fusiform rust fungus (CQF). The inoculum was prepared by collecting aeciospores from three local loblolly pine trees containing naturally sporulating fusiform rust galls. Aeciospores were suspended in sterile distilled water and a spore suspension of 5×10^3 spores ml^{-1} was achieved. This spore suspension was placed into a sterile glass mist atomizer bottle and the uppermost leaf on each of ten seedlings per chamber was sprayed to runoff. Seedlings were then held in a growth chamber at 25°C and 100% relative humidity for 24 h at which time they were returned to the open top chambers (ten seedlings per chamber). To serve as controls, an additional three seedlings per chamber were sprayed to runoff with sterile distilled water and held as with the inoculated seedlings, then returned to the open top chambers. The presence of uredia and telia were assessed at 8, 9, 11, 16, and 19 days after inoculation. This study was repeated beginning 2 days after the initial test.

Pitch canker study: pine

Seeds of loblolly pine from the same mixed lot as those used in the fusiform rust—loblolly pine study were scarified, germinated, and transplanted as for the fusiform rust study. Seedlings were held in the glasshouse over the winter and placed into the open top CO_2 exposure field chambers using the same experimental design employed for the fusiform rust—loblolly pine study. Thirteen seedlings in each chamber were exposed to the two concentrations of atmospheric CO_2 for 6 weeks, at which time the pines had developed new succulent apical growth (candles).

Seedlings were inoculated with a conidiaspore suspension of the pitch canker fungus *Fusarium circinatum* Nirenberg and O'Donnell (FC) [=*F. subglutinans* (Wollenw and Reinking) Nelson et al. f.sp. *pini* (Correll et al.)]. The inoculum was prepared by reisolating the fungus from infected loblolly pine seedlings onto potato dextrose agar (PDA) in sterile petri plates. Following maintenance on PDA for 10 days, 10 ml sterile deionized water was added to ten PDA plates, plates were scraped with a rubber spatula and the resulting suspension poured through two layers of sterile cheesecloth into a 250 ml sterile flask. This process was repeated and the spore suspension was quantified using a hemacytometer and adjusted to 10^6 spores/ml. Inoculations were made using a 5 cm^3 syringe with a 21 gauge needle. The needle was used to create a small wound, 2–4 mm in length, approximately 4 cm below the most recent terminal whorl of each seedling. A small (approximately 5 μl) droplet of quantified inoculum or sterile deionized water was injected into each wound. Ten seedlings per chamber were inoculated with FC; the remaining three per chamber, which served as controls, were inoculated with sterile water. The percentage of seedlings infected was recorded as was the size (length) of each pitch canker lesion beginning 2 weeks after inoculation and continuing weekly up to 7 weeks after inoculation for a total of six assessments. This study was repeated beginning 1 week after the initial test.

In each study, data were averaged for each open top chamber prior to analysis. Data analysis was conducted using the mixed model procedures (Proc Mixed) of the Statistical Analysis System (Littell et al. 1996). Error terms appropriate to the randomized block design ($df = 9$) were used to test the significance of CO_2 concentration. In all cases, differences were considered significant at the $\alpha \leq 0.05$ and trends were recognized at $0.05 < \alpha \leq 0.15$.

Results

Control seedlings were included in each experiment as a check on occurrence of natural infection (i.e., that occurring by means other than the experimental inoculations). In no experiment did any of the control seedlings develop signs or symptoms of disease. Also, in almost all experiments the initial run had higher disease incidences (% seedlings infected) than did the second (replicate) run; therefore, data from the two runs of each experiment are presented separately.

Fusiform rust study: pine

In both runs of the experiment, 6-weeks of growth under elevated CO_2 resulted in significantly taller loblolly pine seedlings (Table 1). Since seedlings were assigned to open top chambers at random, there was no difference in seedling height before placement into the chambers. Therefore, the increase in height was due to an increase in size of the new, succulent, apical growth (candles). Seedling groundline diameters were also statistically larger (run 2) or tended to be larger (run 1) when exposed to elevated CO_2 (Table 1).

Despite an increase in seedling size and the amount of succulent tissue available for infection, percent infection was not significantly affected by CO_2 concentration (Table 1). It should be noted that, in both runs of the experiment, growth under elevated CO_2 resulted in numerically lower (14 and 5.6% for the first and second runs, respectively) percentages of loblolly pine seedlings infected with the fusiform rust fungus; the fact that these differences were not statistically significant was due to high chamber-to-chamber variability. Even though percent infection was not significantly affected by CO_2 concentration, the percentage of loblolly pine seedlings which died as a result of rust infection (rust associated mortality or RAM) was generally significantly lower under elevated CO_2 in both

Table 1 The effects of ambient (365 μmol mol^{-1}) or elevated (720 μmol mol^{-1}) CO_2 on growth and disease variables for loblolly pine (*Pinus taeda*) seedlings inoculated with the fusiform rust fungus (*Cronartium quercuum* f.sp. *fusiforme*) for each run of the experiment

Run	Parameter	Ambient CO_2	Elevated CO_2	*P* values	LSD
1	Height (cm)	23.1	26.2	0.020	2.2
	Diameter (mm)[a]	3.9	4.2	0.124	0.4
	Infection (%)	46.4	32.4	0.170	23.2
	RAM (%)[b]	27.8	15.9	0.048	11.8
	Gall length (mm)	10.0	10.4	0.742	3.4
	Gall diameter (mm)	5.2	5.4	0.413	0.6
2	Height (cm)	23.9	26.1	0.039	2.1
	Diameter (mm)	4.6	5.1	0.009	0.3
	Infection (%)	28.5	22.9	0.515	17.8
	RAM (%)	19.2	5.0	0.130	22.5
	Gall length (mm)	11.5	10.5	0.678	4.9
	Gall diameter (mm)	5.1	4.7	0.654	2.0

Means with associated separation statistics are shown

[a] Diameters were measured at the ground line

[b] RAM is rust associated mortality (i.e., seedlings which died due to infection by the fusiform rust fungus) as a percentage of the total number of seedlings inoculated

runs of the experiment (Table 1). Atmospheric CO_2 concentration had no effect on measures of disease severity (fusiform rust gall length or diameter) in either run of the experiment (Table 1).

Fusiform rust study: oak

In the first run of this experiment, uredia were present on oak leaves at the first evaluation date, 8 days after inoculation; uredia did not appear on any oak leaves until 11 days post-inoculation in run 2 (Fig. 1). The percent of oak seedlings with uredia was consistently lower for seedlings exposed to elevated CO_2 in both runs of the experiment; however, these differences were not statistically significant until 11 or 16 days after inoculation (runs 1 and 2, respectively). The average latent period for uredia (time from inoculation until sporulation) was increased by growth in elevated CO_2 by almost 3 days in run 1 (13.8 vs. 16.6 for ambient and elevated CO_2 treatments, respectively; $P = 0.044$) and by 2 days in run 2 (16.7 vs. 18.8 for ambient and elevated CO_2 treatments, respectively; $P = 0.073$).

Telia, which develop from uredia, did not appear until 11 days after inoculation in both runs of the experiment. The percent of oak seedlings with telia was significantly lower for seedlings exposed to elevated CO_2 at the 16 and 19 days evaluations in both runs of the experiment (Fig. 2). The average latent period for telia was increased by growth in elevated CO_2 by 2 days in run 1 (16.7 vs. 18.7 for ambient and elevated CO_2 treatments, respectively; $P = 0.018$) and in run 2 (16.8 vs. 18.8 for ambient and elevated CO_2, respectively; $P = 0.011$).

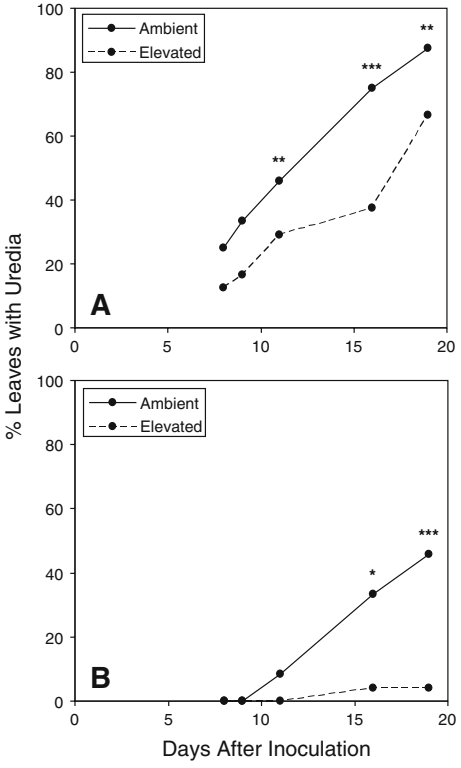

Fig. 1 Percent of northern red oak (*Quercus rubra*) seedlings inoculated with the fusiform rust fungus (*Cronartium quercuum* f.sp. *fusiforme*) which developed uredia over the course of the evaluation period (19 days from inoculation). **A** is the first (initial) run of the experiment and **B** is the second (replicate) run. *Asterisks* (*) represent significant difference between the ambient ($\sim$360 µmol mol^{-1}) and elevated ($\sim$720 µmol mol^{-1}) CO_2 treatments as follows: * = $0.100 < P \leq 0.150$; ** = $0.050 < P \leq 0.100$; *** = $P \leq 0.050$

Pitch canker study: pine

The percent of loblolly pine seedlings which developed cankers following inoculation with the pitch canker fungus was consistently lower ($P < 0.05$) for seedlings grown under elevated CO_2 in both runs of the experiment (Fig. 3). In both runs, disease progressed in a similar fashion in both CO_2 treatments, with infection in elevated CO_2-grown seedlings remaining about half that of ambient-grown seedlings throughout the 7 weeks evaluation period. There were no differences in canker length between the CO_2 treatments at any date in either run of the experiment (data not shown). In both runs, canker length tended to remain small, averaging 4–6 mm beyond the inoculation wound. Despite this small vertical expansion of symptomatic tissue, cankers—when present—generally circumscribed the stem and resulted in death of the apical portion of the seedlings.

Discussion

The fact that disease incidence was lower in the second run of the oak study was likely due to the 2 day differential in inoculation date. Oak leaves are only susceptible to infection by CQF for a short time when they are newly expanding and succulent (Tainter and Baker 1996). Given that seedlings used in both runs of the experiment were germinated at the same time, it is possible that the 2 day difference between runs resulted in a level of leaf maturation that rendered them less susceptible to infection. Despite a 1 week delay in

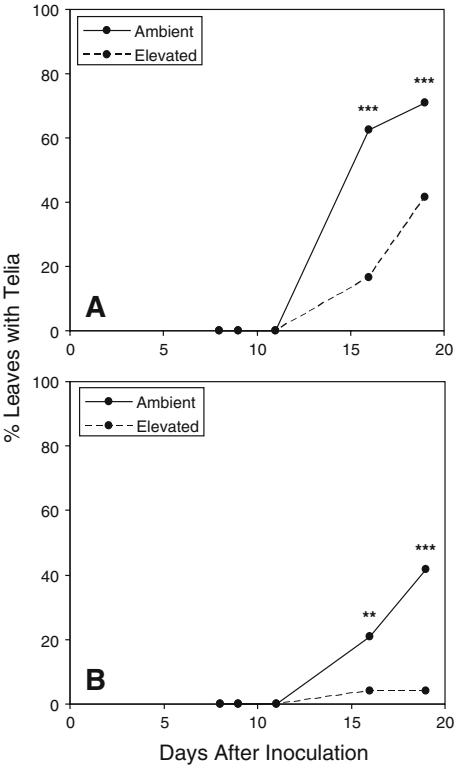

Fig. 2 Percent of northern red oak (*Quercus rubra*) seedlings inoculated with the fusiform rust fungus (*Cronartium quercuum* f.sp. *fusiforme*) which developed telia over the course of the evaluation period (19 days from inoculation). **A** is the first (initial) run of the experiment and **B** is the second (replicate) run. *Asterisks* (*) represent significant difference between the ambient ($\sim$360 µmol mol^{-1}) and elevated ($\sim$720 µmol mol^{-1}) CO_2 treatments as follows: * = $0.100 < P \leq 0.150$; ** = $0.050 < P \leq 0.100$; *** = $P \leq 0.050$

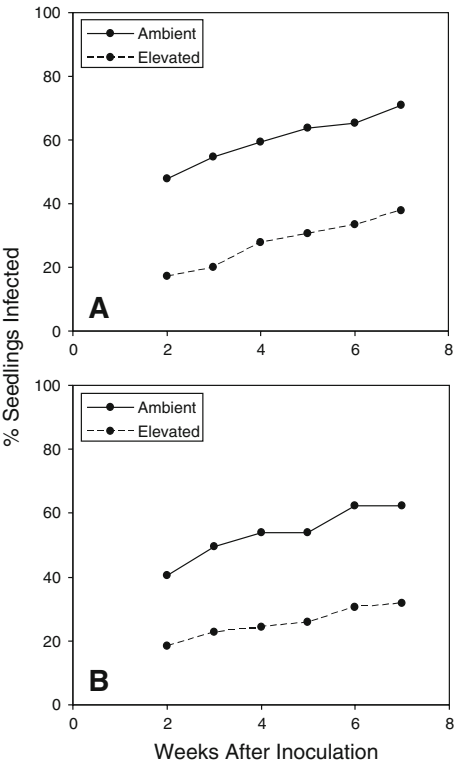

Fig. 3 Percent of loblolly pine (*Pinus taeda*) seedlings inoculated with the pitch canker fungus (*Fusarium circinatum*) which developed cankers over the course of the evaluation period (7 weeks from inoculation). **A** is the first (initial) run of the experiment and **B** is the second (replicate) run. For all evaluation dates in both runs of the experiment, the ambient ($\sim$360 µmol mol^{-1}) and elevated ($\sim$720 µmol mol^{-1}) CO_2 treatments differed at $P \leq 0.050$

inoculation, differences between the two runs of the pitch canker experiment were small. Since pitch canker is caused by an obligate, wound pathogen, it was not surprising that one added week of maturation had little effect on disease incidence.

The fact that fusiform rust infection of loblolly pines was also lower in the second run of the experiment cannot be explained by a delay in inoculation. Fusiform rust galls take months to develop and the two runs of this experiment occurred in subsequent years. An attempt was made to closely replicate the procedures and timing of events (germination, initiation of CO_2 exposure, and inoculation) between the two runs of the experiment. However, it is possible that the lower infection noted in the second run of this experiment was due to differences occurring between the 2 years. For example, even though loblolly pine seeds were obtained from the same forest seed orchard in both years, it is possible that a different mixture of genetic material was used in the two runs of the experiment. Also, although basidiospores were collected from oak leaves in the same manner in both years and the same source of aeciospores was used to infect the oak seedlings in each year (viability exceeded 98% in both years), it is still possible that the inoculum varied in infection efficacy between years.

The null hypothesis tested was: incidence of disease caused by either a facultative or an obligate pathogen (due to an increase in plant growth from CO_2) remains unaffected under elevated atmospheric CO_2. Results indicate that incidence of fusiform rust was statistically unaffected (albeit numerically lower) by growth in elevated CO_2 despite the fact that seedlings were larger (and had additional area for infection). For this obligate pathogen, the hypothesis was not rejected. Despite the fact that size does not explain the lack of

differences in rust incidence, increased growth might explain the decrease in rust associated mortality (RAM) under elevated CO_2; that is, the larger seedlings were better able to live (survive) with the infection.

In contrast to fusiform rust on loblolly pine, the null hypothesis was rejected for the facultative pathogen FC. Pitch canker disease incidence was lower on loblolly pine seedlings grown under elevated CO_2. Further, the fact that oak seedlings had a significant reduction in fusiform rust disease incidence following exposure to elevated CO_2 also rejects the null hypothesis. The reasons for the reductions in disease incidence, regardless of pathogen type, were not determined but are may be related to high CO_2-induced alterations in plant tissue chemistry such as reduction in N concentration (Norby et al. 2001) or increase in C-based defense compounds Pritchard et al. 1997; Runion et al. 1999).

As with disease incidence, it was hypothesized that when given adequate water and nutrition, severity of diseases from facultative and obligate pathogens would remain unaffected under elevated CO_2. In all cases in the present study, the null hypothesis was not rejected since disease severity was unaffected by atmospheric CO_2 concentration. The reason(s) for this lack of a CO_2 effect on disease severity are unclear, but may be related to effects of elevated CO_2 on improvements in overall health of the host plants. Further, with fusiform rust on loblolly pine seedlings, it is possible that gall expansion was maximized (or nearly so) given the size of the seedlings. However, with pitch canker, the reason that cankers did not expand further vertically is anomalous and difficult to explain.

It was interesting that exposure to elevated CO_2 increased the latent period (time from inoculation until sporulation) for both uredia and telia on oak seedlings by 2 days. Fusiform rust has a complex disease cycle and relies extensively on timing such that spore production occurs when the alternate host's tissues (oak leaves for aeciospores and pine tissues for basidiospores) are succulent and more easily infected. A delay of 2 days, as occurred between the two runs of the oak experiment, could have a dramatic impact on this disease cycle.

Given results from the present study, the hypotheses developed regarding the differential effects elevated atmospheric CO_2 will have on diseases caused by obligate versus facultative pathogenic fungi requires reevaluation. Unfortunately, data regarding the effects of atmospheric CO_2 concentration on diseases of crop and forest plant species upon which to base this new assessment remains extremely limited. However, results from the present study suggest that disease incidence—regardless of pathogen type—may be reduced as atmospheric CO_2 concentration continues to rise. Clearly, additional research is required before this can be stated with confidence. Additional research is also needed to elucidate the factors influencing alterations in disease incidence in elevated CO_2 environments.

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References

Agrios GN (1988) Plant pathology, 3rd edn. Academic Press, New York

Amthor JS (1995) Terrestrial higher-plant response to increasing atmospheric [CO_2] in relation to the global carbon cycle. Glob Chang Biol 1:243–274

Ceulemans R, Mousseau M (1994) Effects of elevated atmospheric CO_2 on woody plants. New Phytol 127:425–446

Entry JA, Runion GB, Prior SA, Mitchell RJ, Rogers HH (1998) Influence of CO_2 enrichment and nitrogen fertilization on tissue chemistry and carbon allocation in longleaf pine seedlings. Plant Soil 200:3–11

Graham EA, Nobel PS (1996) Long-term effects of a doubled atmospheric CO_2 concentration on the CAM species *Agave deserti*. J Exp Bot 47:61–69

Hibberd JM, Whitbread R, Farrar JF (1996) Effect of elevated concentrations of CO_2 on infection of barley by *Erysiphe graminis*. Physiol Mol Plant Pathol 48:37–53

Keeling CD, Whorf TP (1994) Atmospheric CO_2 records from the sites in the SIO air sampling network. In: Boden TA, Kaiser DP, Sepanski RJ, Stoss FW (eds) Trends '93: a compendium of data on global change, ORNL/CDIAC-65. The Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, Oak Ridge, pp 16–26

Kimball BA (1983) Carbon dioxide and agricultural yield: an assemblage and analysis of 770 prior observations. WCL Report 14, U S Department of Agriculture, Agricultural Research Service

Kosuge T (1978) The capture and use of energy by diseased plants. In: Horsfall JG, Cowling EB (eds) Plant disease: an advanced treatise, vol III. Academic Press, New York, pp 86–116

Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) SAS system for mixed models. SAS Institute, Cary

Marschner H (1986) Relationship between mineral nutrition and plant diseases and pests. In: Marschner H (ed) Mineral nutrition in higher plants. Academic Press, New York, pp 369–390

Mellilo JM (1983) Will increases in atmospheric CO_2 concentrations affect decay processes? In: Ecosystems center annual report. Marine Biology Laboratory, Woods Hole, pp 10–11

Mitchell RJ, Runion GB, Prior SA, Rogers HH, Amthor JS, Henning FP (1995) Effects of nitrogen on *Pinus palustris* foliar respiratory responses to elevated atmospheric CO_2 concentration. J Exp Bot 46:1561–1567

Norby RJ, Cotrufo MF, Ineson P, O'Neill EG, Canadell JG (2001) Elevated CO_2, litter quality, and decomposition: a synthesis. Oecologia 127:153–165

Poorter H (1993) Interspecific variation in the growth response of plants to elevated CO_2 concentration. Vegetatio 104(105):77–97

Prior SA, Pritchard SG, Runion GB, Rogers HH, Mitchell RJ (1997) Influence of atmospheric CO_2 enrichment, soil N, and water stress on needle surface wax formation in *Pinus palustris* (Pinaceae). Am J Bot 84:1070–1077

Pritchard SG, Peterson CM, Runion GB, Prior SA, Rogers HH (1997) Atmospheric CO_2 concentration, N availability, and water status affect patterns of ergastic substance deposition in longleaf pine (*Pinus palustris* Mill.) foliage. Trees 11:494–503

Rogers HH, Dahlman RC (1993) Crop responses to CO_2 enrichment. Vegetatio 104(105):117–131

Rogers HH, Heck WW, Heagle AS (1983) A field technique for the study of plant responses to elevated carbon dioxide concentrations. J Air Pollut Control Assoc 33:42–44

Runion GB, Curl EA, Rogers HH, Backman PA, Rodriguez-Kabana R, Helms BE (1994) Effects of free-air CO_2 enrichment on microbial populations in the rhizosphere and phyllosphere of cotton. Agric For Meteorol 70:117–130

Runion GB, Entry JA, Prior SA, Mitchell RJ, Rogers HH (1999) Tissue chemistry and carbon allocation in seedlings of *Pinus palustris* subjected to elevated atmospheric CO_2 and water stress. Tree Physiol 19:329–335

Tainter FH, Baker FA (1996) Fusiform rust. In: Tainter FH, Baker FA (eds) Principles of forest pathology. Wiley, New York, pp 518–542

Thomas JF, Harvey CN (1983) Leaf anatomy of four species grown under continuous CO_2 enrichment. Bot Gaz 144:303–309

Thompson GB, Drake BG (1994) Insects and fungi on a C_3 sedge and a C_4 grass exposed to elevated atmospheric CO_2 concentration in open-top chambers in the field. Plant Cell Environ 17:1161–1167

Thompson GB, Brown JKM, Woodward FI (1993) The effects of host carbon dioxide, nitrogen and water supply on the infection of wheat by powdery mildew and aphids. Plant Cell Environ 16:687–694

Torbert HA, Prior SA, Rogers HH, Wood CW (2000) Review of elevated atmospheric CO_2 effects on agro-ecosystems: residue decomposition processes and soil carbon storage. Plant Soil 224:59–73

Yelle S, Beeson RC Jr, Trudel MJ, Gosselin A (1989) Acclimation of two tomato species to high atmospheric CO_2. 1. Starch and sugar concentrations. Plant Physiol 90:1465–1472